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Two-Dimensional Correlation Analysis of Visible/Near-Infrared Spectral Intensity Variations of Chicken Breasts with Various Chilled and Frozen Storages

YONGLIANG LIU, FRANKLIN E. BARTON, II,* BRENDA G. LYON, WILLIAM R. WINDHAM, AND CLYDE E. LYON

Richard B. Russell Research Center, Agricultural Research Service, U.S. Department of Agriculture, Athens, Georgia 30604-5677

Generalized two-dimensional (2D) correlation analysis of visible/near-infrared (NIR) spectra was performed to characterize the spectral intensity variations of chicken muscles induced by either storage time/temperature regime or shear force values. The results showed that intensities of two visible bands at 445 and 560 nm increase with the storage temperature under identical treatment, possibly indicating a color change due to frozen storage. The 2D NIR correlation spectra indicated that all NIR bands reduce their spectral intensities, probably due to the water loss and compositional alterations during the freeze−thaw process as well as the tenderization development in muscle storage. The heterospectra correlating the spectral bands in both visible and NIR regions exhibited a strong correlation and suggested the sequential change between color and other developments in muscles. In addition, shear value-induced NIR spectral intensity variations detected significant differences in spectral features between tender and tough muscles.

KEYWORDS: Two-dimensional correlation analysis; visible/NIR spectroscopy; chicken muscles; myoglobin; tenderness

INTRODUCTION

Processed chicken carcasses are regularly marketed as fresh refrigerated product (soft flesh), as chill pack product (hard on surface and soft deep muscle tissues), and as frozen product (hard frozen throughout). After the consumer has purchased any of these product types, they are usually kept in the refrigerator or placed in the freezer. All of these different temperature scenarios will cause several changes in meat that can affect its quality. These changes are reflected in many characteristics such as color, texture, flavor, and microbial profile. Also, the characteristics of meat are influenced greatly by a variety of factors, for example, specific muscle species, sex, pH value, and period of post-mortem storage (*1*, *2*).

Visible/near-infrared (NIR) spectroscopy has been developed and applied widely in safety and quality control issues of chicken meat products. The applications include the quantitative prediction of the physical characteristics of heat-treated chicken patties (*3*, *4*), the classification differentiation of "slow-growing" chickens from "industrial" ones (*5*), the discrimination of "fresh" and "frozen" chicken meat (*6*), and the classification of chicken carcasses into wholesome and unwholesome classes at the slaughter plant (*7*, *8*).

Moreover, generalized two-dimensional (2D) correlation analysis was applied to investigate the visible/NIR spectral features of chicken meat under a variety of conditions, such as cooking, thawing, and cold storage as well as diseases (*9*−*12*). Results have shown that the 2D visible/NIR approach can not only establish the spectral band assignments but also monitor the complex sequence of events arising from the changes in meat. Furthermore, the visible bands identified through the 2D studies have been found to be useful as an indicator of meat color variation during cooking, irradiation, and cold storage (*13*, *14*). The results have developed the relationship between spectral absorption and meat color structure and also demonstrated the significance of the 2D approach in analyzing overlapped and broad bands of meat and food products. However, other important meat characteristics, such as eating quality, including instrumental texture and sensory evaluation, have not been included in studies.

The objectives of this study were to investigate the visible/NIR spectral features of chicken muscles with various storage time and temperature treatments and to correlate meat tenderness (the shear force values) with the spectral intensity variations.

MATERIALS AND METHODS

Meat Samples and Storage Treatments. Five hundred and twenty-five mixed sex, 42-day-old, commercially processed broiler carcasses were obtained from a local plant and transported to the laboratory. All carcasses were held on ice at the processing plant at least 8 h prior to pickup. Skinless left and right breast fillets were removed from each carcass and then packaged immediately in polyethylene bags.

* Author to whom correspondence should be addressed [telephone (706) 546-3497; fax (706) 546-3607; e-mail wbarton@qaru.ars.usda.gov].

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The bags were placed in five holding chambers set at temperatures of 4, 0, −3, −12, and −18 °C for 2 days of storage (treatment A) and 7 days of storage (treatment B). Treatment C samples were held at the five temperatures for the same 7 days as treatment B but were then stored for an additional 7 days at −18 °C. Following the thawing at 4 °C for frozen samples after appropriate storage treatments, the left breasts were used for visible/NIR spectral collection and the right ones were used for Warner−Bratzler shear force and sensory analysis.

Cooking and Warner−Bratzler Shear Force Measurement. The right breast samples remained individually bagged through removal from storage for thawing overnight at 4 °C and then cooking in 85 °C agitated water to an end-point temperature of 80−82 °C. When the target temperature was reached after ∼20 min, sample bags were removed from the cooking water and cooled in tap water for 15 min.

Shear values were obtained using a benchtop Warner−Bratzler shear device (Warner−Bratzler shear, Salter model 235, G-R Electrical Manufacturing Co., Manhattan, KS) on the 1.9-cm strip cut from each cooked breast piece. The strip was marked at two points for shearing, and the thickness at each point was measured with calipers so that values could be expressed as force per square centimeter as well as kilograms of force. The strip from each fillet was sheared at each marked point, and the two values were averaged.

Visible/NIR Spectroscopic Measurement. Samples were scanned on a NIRSystems 6500 monochromator (NIRSystems, Silver Spring, MD) equipped with a sample transport module and a half-coarse sample cell. Reflectance measurements were recorded over the 400−2498 nm wavelength range at 2 nm intervals and 32 scans. The instrument was operated by the software package NIRS3 v.4.10 (Infrasoft International, Inc., Port Matilda, PA).

After treatments A and B (storage for 2 and 7 days), samples from 4 °C were scanned immediately, and breasts from 0, −3, −12, and −18 °C were allowed to equilibrate to 4 °C and then scanned. Samples from storage treatment C were tempered back to 4 °C and scanned.

The obtained visible/NIR spectra were transformed into .spc files (Grams file format), were simply offset to zero, and then were smoothed (Savitzky−Golay smoothing function and 11 smoothing points) with the use of Grams/32 software (Galactic Industrious Corp., Salem, NH). Each average spectrum was obtained by averaging the spectra of 35 samples in individual storage treatments (A, B, or C) and temperatures (4, 0, −3, −12, or −18 °C) by using the Grams/32; eventually 15 average spectra were available for further analysis. To examine the sequential change of spectral intensity variations with the consideration of meat tenderness, 10−50 spectra that had the measured shear force values in the respective ranges of <3.0, 3.0−4.0, 4.0−5.0, 5.0−6.0, 6.0−7.0, 7.0−8.0, 8.0−9.0, 9.0−10.0, and >10.0 kg were averaged, and a new data set with the ordered sequence of increasing tenderness was subjectively created.

2D Correlation Analysis. The 2D correlation analysis was performed using the KG2D correlation program developed by Ozaki et al. of the School of Science, Kwansei-Gakuin University, Japan. In the 2D approach, dynamic (or difference) spectra, obtained by subtracting the average spectrum of the individual set from each spectrum, were used to develop the generalized 2D correlation spectra.

Selection of a minimum threshold level of the contour map is somewhat arbitrary. Some of the fine features in the correlation spectrum could be omitted if the threshold is too high, whereas minor features arising from noise and baseline distortions might be overaccentuated if the selected threshold is too low. The threshold of each contour line map was set to 15% of the maximum point of the map.

The generalized 2D correlation spectra consist of synchronous and asynchronous correlation spectra. A synchronous 2D correlation spectrum characterizes the similarity between the sequential variations of spectral intensities. Autopeaks located at the diagonal position represent the extent of dynamic variations of spectral intensity at different wavelengths. Synchronous cross-peaks appear at off-diagonal positions if the basic trends of dynamic variations observed at two different wavelengths of the cross-peak spectral coordinate are similar. Positive cross-peaks (shown in solid lines) indicate that intensities at both wavelengths are either increasing or decreasing together, whereas negative peaks (shown in dashed lines) mean that one intensity is increasing and the other decreasing.

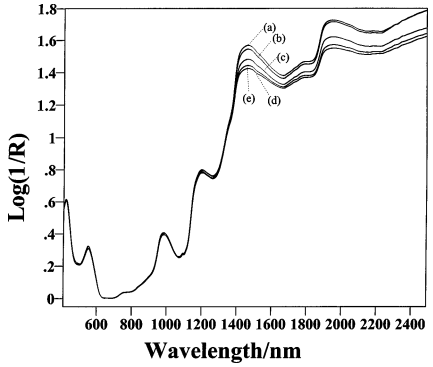

Figure 1. Average visible/NIR reflectance spectra in the 410–2490 nm region of chicken breasts from treatment C (7 days at 4, 0, –3, –12, and –18 °C followed by 7 additional days at –18 °C): (**a**) 4 °C; (**b**) 0 °C; (**c**) –3 °C; (**d**) –12 °C; (**e**) –18 °C.

An asynchronous 2D correlation spectrum consists exclusively of off-diagonal cross-peaks. It characterizes the difference between the perturbation-dependent sequential variations of spectral intensities. Asynchronous cross-peaks appear if the basic trends of dynamic variations observed at two different wavelengths of the cross-peak spectral coordinate are dissimilar. From the sign of an asynchronous cross-peak, it is possible to assign the specific sequence of events occurring at different temperatures/perturbation variables. A negative cross-peak indicates that the spectral intensity change observed at λ_1 occurs after that at λ_2, and positive peaks indicate the opposite.

RESULTS AND DISCUSSION

Visible/NIR Spectra of Chicken Breast Muscles at Various Storage Regimes. Figure 1 shows the representative reflectance spectra in the spectral region of 410−2490 nm of the chicken breast muscles from treatment C (7 days at 4, 0, −3, −12, and −18 °C followed by 7 additional days at −18 °C). In this study, the carcasses were well-bled, and thus myoglobin is the primary heme pigment in the muscles (*1*, *2*, *15*). Two broad visible bands centered at 430 and 560 nm are associated with various forms of myoglobin (*1*, *2*, *9*). The band at 980 nm is commonly thought to be the second overtones of the O−H stretching mode of water. Features between 1100 and 1250 nm are from the second overtones of the C−H stretching modes, and their first overtones appear in the 1600−1850 nm region (*16*). Bands in the 1250−1400 nm region are ascribed to combination bands of the C−H vibrations. Broad and intense bands in the 1400−1600 nm region are due to the overlaps of the first overtones of the O−H/N−H stretching modes of self-associated and water-bonded OH/NH groups in meat composition. The bands in the 1850−2490 nm region arise from the combination modes of different vibrations of C−H, O−H, and N−H as well as other functional groups (such as C═O).

In **Figure 1**, it is noted that the intensities of two visible bands at 430 and 560 nm increase but are insignificant, whereas those of NIR bands at higher wavelength (>1460 nm) decrease clearly, with the storage temperature. The frozen temperature related visible/NIR spectral intensity variations suggest possible color, physical, and chemical changes in chicken muscles and form a basis for the classification of stored muscles into "fresh" and "frozen" classes with great success (*6*).

2D Correlation Analysis of Chicken Breast Muscles at Various Storage Regimes. Two-dimensional correlation analysis of visible/NIR spectral intensity variations of chicken muscles with three storage treatments (A, B, or C) was developed, respectively. The results from the spectral set of treatment C provided the better feature and were taken for further analysis.

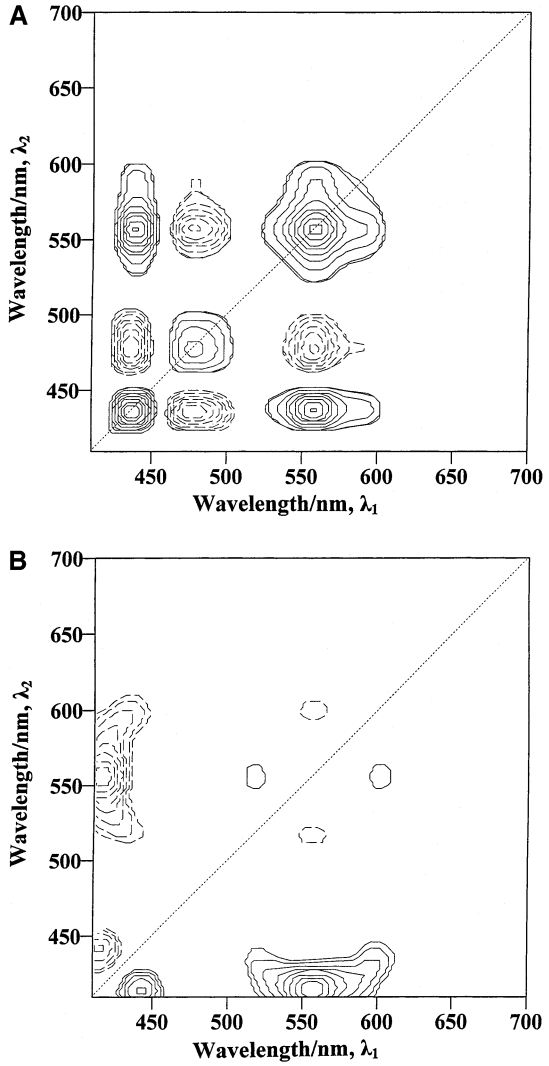

Figure 2. 2D correlation spectra of chicken breasts from treatment C in the 410–700 nm visible region: (**A**) synchronous contour line version; (**B**) asynchronous contour line version.

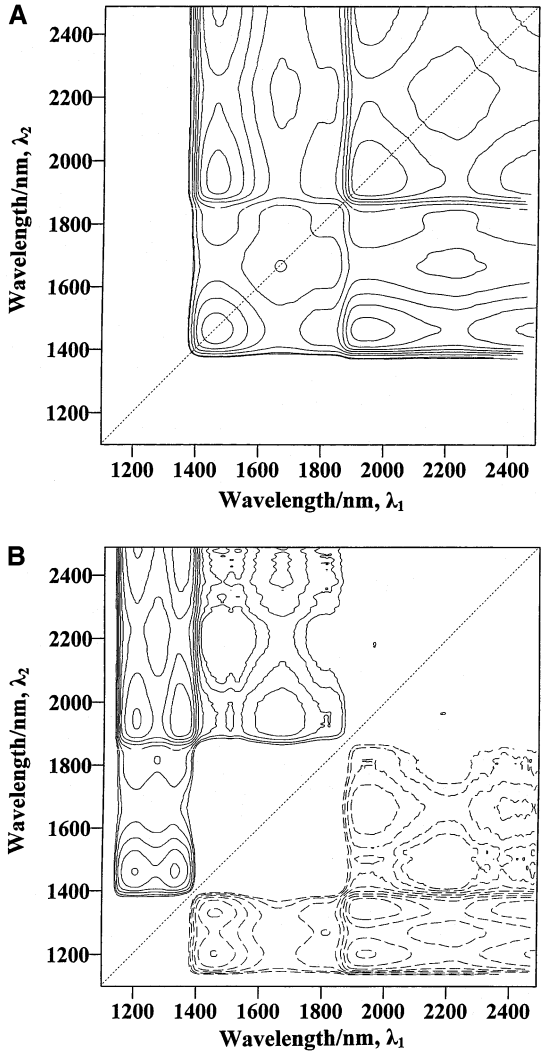

Figure 3. 2D correlation spectra of chicken breasts from treatment C in the 1100–2490 nm NIR region: (**A**) synchronous contour line version; (**B**) asynchronous contour line version.

Panels **A** and **B** of **Figure 2** show, respectively, synchronous and asynchronous 2D visible correlation spectra of chicken muscles under the condition of treatment C. Dominant autopeaks on the diagonal position are observed around 445, 475, and 560 nm, and cross-peaks associated with the autopeaks are also observed at off-diagonal positions (**Figure 2A**). The appearance of the autopeaks means that the intensities of these bands change very significantly with the storage temperature. The positive cross-peaks marked by the solid lines are found between the 445 and 560 nm bands, whereas negative cross-peaks marked by the dotted lines are observed between the 475 nm band and the 445 and 560 nm bands. This observation, combined with **Figure 1**, indicates that the spectral intensities at 445 and 560 nm increase while that at 475 nm decreases as the storage temperature was lowered. The 445, 475, and 560 nm bands have been assigned to deoxymyoglobin (DeoxyMb), metmyoglobin (MetMb), and oxymyoglobin (OxyMb) species, respectively (*9*). The increase of both DeoxyMb and OxyMb fractions might be linked to the meat redness after the freeze−thaw effect. The results are consistent with an early study on thawing behavior of frozen chicken breast by using visible/NIR spectroscopy and 2D correlation analysis (*10*), in which the peak intensities at 435 and 555 nm increased due to the relaxation of DeoxyMb and OxyMb with the thawing. Another possibility is that meat discoloration occurs relatively faster at higher temperatures (for example, 4 °C) than at lower temperatures during treatment C; hence, the frozen procedure could preserve the meat color.

For the same storage temperature domain, the asynchronous spectrum in **Figure 2B** reveals that both the 445 and 560 nm bands are asynchronously correlated with the band at 415 nm, a Soret band for OxyMb (*17*). Meanwhile, the intensity change of the 560 nm band happens after those of the 515 and 600 nm bands. The 515 and 600 nm bands might be associated with myoglobin derivatives, which are placed in different molecular environments of heme vibrations (*9*) and/or are from different myoglobin oxidization states.

Figure 3A shows the synchronous 2D correlation spectrum in 1100−2490 nm NIR region. It reveals the presence of two autopeaks at 1465 and 1960 nm (the "autopeaks" around 1670 and 2190 nm were actually the valleys that can be seen from synchronous three-dimensional representation), and their intensities decrease with the elevation of frozen temperature. Because the 1465 nm band corresponds to the first overtone of OH−NH stretching modes and the 1960 nm band to combination bands of OH stretching and deformation modes of water, positive cross-peaks correlating the two bands suggest that intensity changes of the two bands are reasonably similar. They

also suggest the obvious reduction of water and protein fraction in muscles subjected to different temperature treatments.

During the period of nonfrozen storage (4 and 0 °C), tenderization of meats develops as a result of protein degradation (proteolysis), which originates within the myofiber and is responsible for the degradation of cell constituents (*1*, *2*). Spectral intensities of all NIR peaks decrease with the storage temperature, probably due to the following reasons: (1) water loss following the melting of ice crystals produced during the frozen stage; (2) structure alterations of meat compositions (such as proteins and lipids) during the freeze−thaw process; and (3) tenderization development during the storage treatment C.

The corresponding asynchronous spectrum (**Figure 3B**) shows the complex clusters of cross-peaks located at the spectral coordinates at 1200, 1330, 1465, 1670, 1960, and 2380 nm. Two main absorption peaks at 1465 and 1960 nm wavelengths, due to the OH−NH vibrations, are accompanied by several asynchronous cross-peaks at 1200 and 1330 nm ascribed to the C−H fractions. The result suggests that the storage temperature induced change of the O−H/N−H vibrations in meats differs from that of C−H modes. The signs of asynchronous cross-peaks indicate that OH/NH fractions vary later than the C−H fractions at 1200 and 1330 nm, suggesting the significant coordination process between hydrophilic OH and NH groups rather than hydrophobic CH groups. **Figure 3B** also indicates that there is an asynchronicity between the 1670 nm band and two bands at 1960 and 2380 nm and that the intensity change of the 1670 nm band occurs before those of the 1960 and 2380 nm bands. Probably, the 1670 nm band is assigned to the first overtone of the CH stretching modes, whereas the 1960 and 2380 nm bands are respectively related to the combinations of OH stretching and deformation modes of water and the second overtone of the OH deformation modes of water.

The synchronous and asynchronous 2D heterospectral correlation, shown in panels **A** and **B**, respectively, of **Figure 4**, are interesting because they compare the absorbance bands in the visible and NIR regions. Positive synchronous cross-peaks indicate that the decrease in the spectral intensity of the 475 nm visible band is clearly correlated with those of the NIR bands at 1465 and 1960 nm, whereas negative cross-peaks suggest the opposition in intensity changes between the two visible bands (445 and 560 nm) and two NIR bands (1465 and 1960 nm). **Figure 4B** reveals the existence of asynchronicity among the storage temperature dependent variations of spectral intensities in these regions. The signs of asynchronous cross-peaks indicate that the intensity increase at the 445 and 560 nm bands proceeds those at 1200, 1330, 1465, and 1960 nm NIR bands. Also, the intensity variation of the 610 nm band occurs later than that of the 1960 nm NIR band. Therefore, the results suggest that the meat color is sensitive to frozen temperature and that change in meat color happens before other processes, such as the loss of water and the development of meat tenderness.

In addition to storage regime induced spectral intensity fluctuations, chicken breasts had clear differences in Warner−Bratzler shear force values with storage temperature. Averages of shear force value in treatment C were 3.96, 4.18, 4.48, 4.19, and 4.17 kg, respectively, for the muscles stored at temperatures of 4, 0, −3, −12, and −18 °C. Generally, the muscles stored at 4 °C required less force to shear than did the muscles with frozen treatment. This is expected and reasonable, as aging-associated chemical, physical, and structural changes of muscles at 4 °C might take place more rapidly than those at lower temperatures.

2D Correlation Analysis of Shear Value Dependent NIR Spectra. Although the above 2D correlation analysis provides

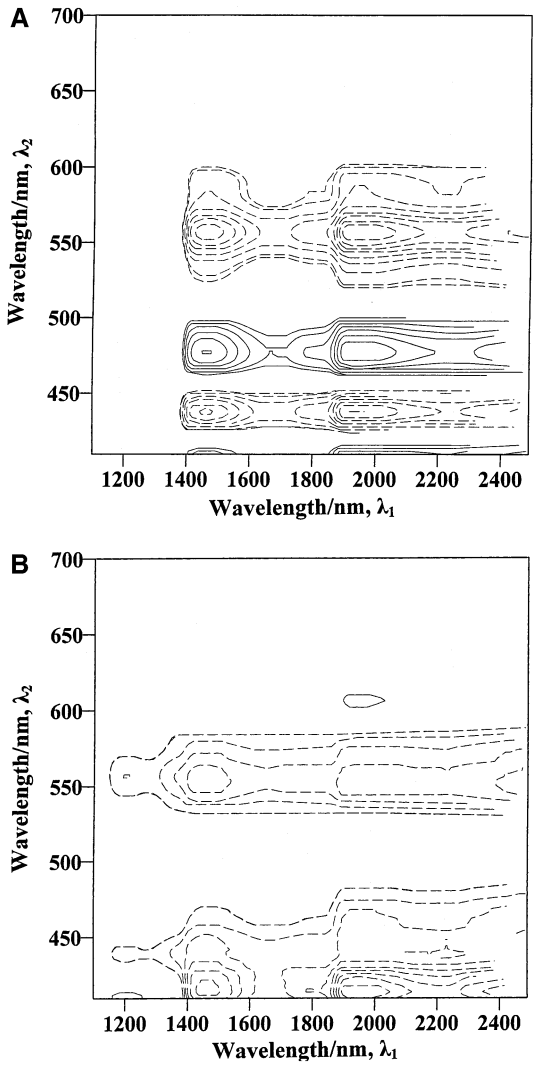

Figure 4. 2D correlation spectra of chicken breasts from treatment C in the off-diagonal region bound by 410–700 nm (vertical) and 1100–2490 nm (horizontal): (**A**) synchronous contour line version; (**B**) asynchronous contour line version.

a general overall view of the spectral intensity variations of chicken muscles corresponding to the storage temperature/time perturbation, it is unclear which chemical and/or physical attributes have the most effect on spectral intensity of the visible/NIR bands. The reason is that 525 chicken muscles were used, and probably samples within the individual treatment had larger variations in tenderness and color characteristics than those undergone during the period of storage time/temperature treatments.

In the following analysis, we sorted all of the spectra on the basis of shear force values and subsequently averaged the spectra having the shear values in the range of <3.0, 3.0−4.0, 4.0−5.0, 5.0−6.0, 6.0−7.0, 7.0−8.0, 8.0−9.0, 9.0−10.0, and >10.0 kg, respectively. Then, we combined the averaged spectra into a new data set with the ordered sequence of increasing shear force values. Such an arrangement allows the monitoring of the sequential change of spectral intensity variation of chicken muscles with the consideration of shear force perturbation solely and with the ignorance of any treatments.

Figure 5 shows the representative NIR spectra of chicken muscles in the 1100−2490 nm region. The spectral feature in the 410−700 nm visible region is not suitable for discussion because of the lack of color reference information. Careful

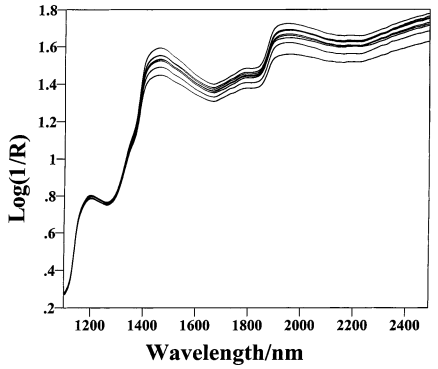

Figure 5. Representative spectra of chicken muscles with different shear values in the 1100–2490 NIR region.

examination indicates that, with an increase in the shear force value, peak intensities of the 1465 and 1960 nm bands gradually increase within the shear range of up to 8.0 kg and then decrease afterward. This observation is in good consistent with Lyon et al.'s previous study on the definition of tender and tough chicken meat, in which a relationship between shear value and sensory panel evaluation was established (*18*). Hereafter, the former range is referred to as tender meat (up to 8.0 kg in shear value) and the latter as tough meat (8.0 kg and above in shear value). Such small ranges were set to accentuate the shear force value induced changes of NIR spectra.

Figure 6A shows the synchronous 2D NIR correlation spectrum of tender meat. It reveals two autopeaks at 1465 and 1960 nm ("autopeaks" near 1710 and 2300 nm were valleys), increasing in intensity with the shear value. The corresponding asynchronous spectrum (**Figure 6B**) shows several cross-peaks at 1120, 1275, 1450, 1960, 2000, 2230, and 2430 nm, and the signs indicate that OH/NH fractions (1450 and 1960 nm) vary after the C−H fractions (1120 and 1275 nm). Also, the 1450 nm band changes its intensity later than the bands around 2000, 2230, and 2430 nm.

There is no noticeable distinction between **Figure 7A**, a 2D NIR correlation spectrum of tough meats, and **Figure 6A**; probably variation in water amount among tender or tough meats predominates and superposes other components. However, spectral intensities of the 1465 and 1960 nm bands in **Figure 7A** decrease with increase in shear value. The asynchronous spectrum depicted in **Figure 7B** reveals that the intensity changes at the 1440 and 1860 nm bands occur earlier than those of other bands at 1960, 2300, and 2430 nm.

Up to this point, 2D NIR correlation spectra elucidate the differences in spectral features between tender and tough meat. For example, there are at least eight bands at 1120, 1275, 1450, 1465, 1960, 2000, 2230, and 2430 nm in tender meat and six bands at 1440, 1465, 1860, 1960, 2300, and 2430 nm in tough meat. Characteristic NIR bands of tender and tough meats are summarized in **Table 1**, which also reveals that there are numerous OH, NH, and CH as well as other groups in meats, which are reasonably placed in very different molecular structures and surroundings. For example, the 1440 and 1450/1465 nm bands might be assigned to the first overtones of OH stretching modes for self-associated water species and water complexes (i.e., the interaction of water with proteins through hydrogen bonding; *10*).

Undoubtedly, the NIR bands discussed above include the contributions from other meat components. For example, cell membrane phospholipids may absorb at the observed O−H and N−H wavelengths, and the peptide backbone of proteins

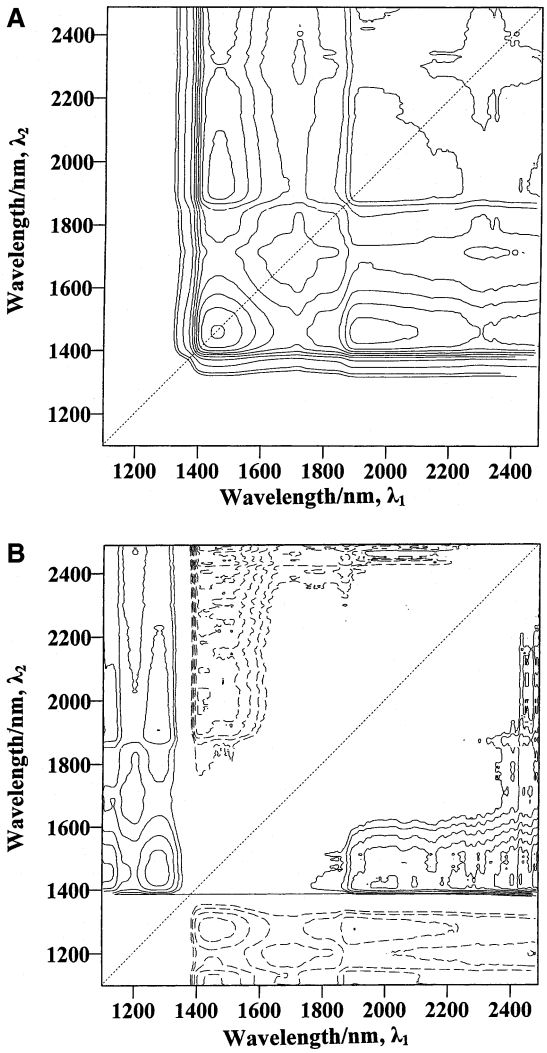

Figure 6. 2D correlation spectrum of tender chicken breasts in the 1100–2490 nm NIR region: (**A**) synchronous contour line version; (**B**) asynchronous contour line version.

contributes to C−H bands. However, in general, the effects on spectral characterization are minimal due to their low fractions.

CONCLUSIONS

The obtained results demonstrate the usefulness of 2D visible and NIR correlation spectroscopy in understanding the chemistry associated with the storage regime of chicken muscles and their physical characteristics. Analysis of the 2D spectral variations of frozen temperature dependent muscles in the visible region reveals at least three absorption bands around 445, 475, and 560 nm assignable to DeoxyMb, MetMb, and OxyMb species, respectively. With the frozen temperature, peak intensities at the 445 and 560 nm bands increase, suggesting the possibility of preserving color appearance in frozen meats.

Two-dimensional NIR observation indicates the intensity reduction for all NIR peaks, probably due to water loss and compositional alterations during the freeze−thaw process as well as tenderization development in meat storage. Six characteristic bands at 1200, 1330, 1465, 1670, 1960, and 2380 nm are due to CH, OH/NH, and other groups.

Meanwhile, strong correlations exist between the visible and NIR bands, which suggest the sequential change between color and other developments, such as water loss and tenderization.

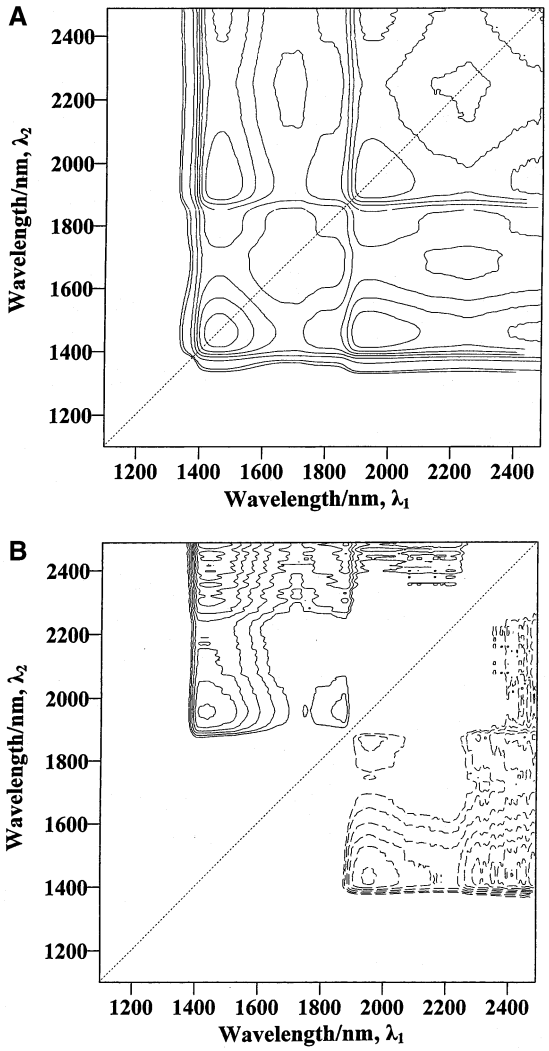

Figure 7. 2D correlation spectrum of tough chicken breasts in the 1100–2490 nm NIR region: (**A**) synchronous contour line version; (**B**) asynchronous contour line version.

Table 1. Characteristic NIR Wavelengths of Tender and Tough Poultry Meat Samples from 2D Correlation Analysis

	wavelength (nm)	
	tender meat	tough meat
synchronous	1465	1465
	1960	1960
asynchronous	1120	
	1275	
		1440
	1450	
		1860
	1960	1960
	2000	
	2230	
		2300
	2430	2430

The result obtained from 2D correlation synchronous and asynchronous spectra indicates the significant differences in spectral features between tender and tough meats, which could not be observed from conventional one-dimensional spectra; that is, meat tenderness is influenced by more than one component.

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